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Infarct Size Reduction by AT_1-Receptor Blockade Through a Signal Cascade of AT_2-Receptor Activation, Bradykinin and Prostaglandins in Pigs

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Objective. **We studied the effect of the angiotensin II type 1 (AT_1)-receptor antagonist candesartan on infarct size resulting from regional myocardial ischemia in pigs.**

Background. **The effects of AT_1-receptor blockade on infarct size in different species remain controversial and its potential cardioprotective mechanisms are still unclear. In pigs, infarct development closely resembles that observed in humans.**

Methods. **A total of 62 enflurane-anesthetized pigs underwent a protocol of 90-min low-flow ischemia and 120-min reperfusion. Systemic hemodynamics (micromanometer), regional myocardial function (sonomicrometry), regional myocardial blood flow (microspheres) and infarct size (TTC [triphenyl tetrazolium chloride]-staining) were determined.**

Results. **Left ventricular peak pressure decreased with candesartan (1 mg/kg i.v.) from 97 ± 2 standard error of the mean (SEM) to 86 ± 5 mm Hg and was then readjusted by aortic banding. In placebo pigs (n = 9), infarct size was 21.8 ± 4.8% of the area at risk. Candesartan (n = 7) reduced infarct size to 9.7 ± 2.5% ($p < 0.05$). Pretreatment with the AT_2-receptor antagonist PD123319 (3 μg/kg/min intracoronarily [i.c.]; n = 8), the bradykinin B_2-receptor antagonist HOE140 (0.01 μg/kg/min i.c.; n = 8) or the cyclooxygenase inhibitor indomethacin (10 mg/kg i.v.; n = 8) per se did not affect infarct size but did abolish the reduction of infarct size achieved by candesartan (PD123319 + candesartan (n = 7): 23.2 ± 4.7%; HOE140 + candesartan (n = 7): 18.2 ± 4.0%; indomethacin + candesartan (n = 8): 21.1 ± 5.2%). Hemodynamics, regional myocardial blood flow during ischemia and the area at risk were comparable among all groups of pigs.**

Conclusions. **Reduction of infarct size by the AT_1-receptor antagonist candesartan in pigs involves angiotensin II type 2 receptor (AT_2) activation, bradykinin and prostaglandins.**

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The cardiovascular effects of angiotensin II have been largely attributed to activation of the angiotensin II type 1 (AT_1) receptor (1). Indeed, angiotensin II is locally formed in ischemic dog hearts (2), and AT_1-receptor blockade increases coronary blood flow during ischemia in dogs (3), improves functional and metabolic recovery after myocardial ischemia in rats (4,5), reduces ischemia-related arrhythmias in rats (6,7) and guinea pigs (8), and finally attenuates ventricular dilatation after myocardial infarction in rats (6,9).

The AT_1-receptor antagonist losartan (DuP753) reduced infarct size in pigs (10), but not in rats (11–13), rabbits (14–16) and dogs (17). The AT_1-receptor antagonist candesartan cilexetil (TCV116), the prodrug of candesartan, reduced creatine kinase release following 30 min of global ischemia in isolated rat hearts (5). Because infarct size studies in large animals with candesartan are lacking and the potential mechanisms involved in such cardioprotection are unclear, we studied the effect of the AT_1-receptor antagonist candesartan on infarct size resulting from severe regional myocardial ischemia in enflurane-anesthetized pigs. Studies were performed in pigs, as in this species coronary anatomy (18), the extent of collateral flow (19) and time course of infarct development most closely resemble that observed in humans (20).

Blockade of the AT_1-receptor increases the angiotensin II concentration (21), which may then activate the angiotensin II type 2 (AT_2)-receptor (7,9). The AT_1-receptor activation, in turn, increases the formation of local kinins in isolated dog coronary arteries (22). Left ventricular (LV) dilatation following myocardial infarction in rats was attenuated by AT_1-receptor blockade (9); this protective effect was, however, abolished by blockade of either the AT_1-receptor or the bradykinin B_2-receptor. Therefore, a kinin-mediated mechanism secondary to activation of the AT_2-receptor may contribute to the cardioprotection achieved by AT_2-receptor blockade. Consequently, we tested in subsequent steps of the experimental protocol the effect of candesartan on infarct size after pretreatment with the AT_2-receptor antagonist

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Abbreviations and Acronyms

ACE = angiotensin-converting-enzyme
AT_1 = angiotensin II type 1 receptor
AT_2 = angiotensin II type 2 receptor
LAD = left anterior descending coronary artery
LV = left ventricular
SEM = standard error of the mean
TTC = triphenyl tetrazolium chloride

PD123319 (23) and with the bradykinin B_2-receptor antagonist HOE140 (24).

Because the beneficial effect of angiotensin-converting-enzyme (ACE) inhibition on the recovery of stunned myocardium also involves bradykinin and subsequently prostaglandins (25), we finally used the cyclooxygenase inhibitor indomethacin (26) to study the involvement of prostaglandins in the beneficial effect of candesartan.

Methods

The experimental protocols employed in this study were approved by the Bioethical Committee of the district of Düsseldorf and they adhere to the guiding principles of the American Physiological Society.

Experimental model. Sixty-four Göttinger minipigs (20 to 40 kg) of either sex were initially sedated using ketamine hydrochloride (1 g intramuscularly [i.m.]) and then anesthetized with thiopental (Trapanal, 500 mg intravenously [i.v.]). Through a midline cervical incision, the trachea was intubated for connection to a respirator (Dräger, Lübeck, Germany) equipped with an enflurane vaporizer. Anesthesia was then maintained throughout the study using enflurane (1% to 1.5%) with an oxygen/nitrous oxide mixture (40%:60%). Through the cervical incision, both common carotid arteries and internal jugular veins were isolated. The arteries were cannulated with polyethylene catheters, one for the measurement of arterial pressure, the other to supply blood to the extracorporeal circuit. The jugular veins were cannulated for volume replacement using 0.9% NaCl and for the return of blood to the animal from the coronary venous line (see below). A left lateral thoracotomy was performed in the fourth intercostal space and the pericardium opened. A micromanometer (P7, Konigsberg Instruments, Pasadena, California) was placed in the left ventricle through the apex together with a saline-filled polyethylene catheter (used to calibrate the micromanometer in situ) and secured with a purse-string suture. Ultrasonic dimension gauges were implanted in the LV myocardium to measure the thickness of the anterior and posterior (control) wall (System 6, Triton Technologies, San Diego, California). Arterial blood gases were monitored frequently in the initial stages of the preparation until stable and then periodically throughout the study (ABL 510 Radiometer, Copenhagen, Denmark). The proximal left anterior descending (LAD) coronary artery was dissected over a distance of 1.5 cm, ligated, cannulated and the distal LAD perfused from an extracorporeal circuit.

Prior to coronary cannulation the pigs were anticoagulated with 20,000 IU sodium heparin; additional doses of 10,000 IU were given at hourly intervals. The system included a roller pump, windkessel and two side ports, one for the injection of radiolabeled microspheres, the other for intracoronary infusion of drugs. Coronary arterial pressure was measured from the sidearm of a polyethylene "T"-connector (Cole-Parmer, Chicago, Illinois) used as catheter tip with an external transducer (DPT-6003, pvb Medizintechnik GmbH, Kirchseeon, Germany). The large epicardial vein parallel to the LAD was dissected and cannulated. Coronary venous blood was drained to an unpressurized reservoir and then returned to a jugular vein using a second roller pump. Heart rate could be controlled throughout the study by left atrial pacing (Hugo Sachs Elektronik Type 215/T, Hugstetten, Germany) and LV peak pressure could be adjusted by aortic banding. The completed preparation was allowed to stabilize for at least 30 min before control measurements were made.

Under control conditions the arterial perfusion pump was adjusted so that the minimum coronary arterial pressure did not fall below 70 mm Hg to avoid an initial hypoperfusion. Therefore, mean coronary arterial pressure exceeded peak LV pressure. This preparation retains a dynamic coronary autoregulation, demonstrating the lack of excessive anesthetic-dependent coronary vasodilation (27). Rectal temperature was monitored and kept between 37 to 38°C by use of a heated surgical table and drapes.

Regional myocardial function. End-diastole was defined as the point when LV dP/dt started its rapid upstroke after crossing the zero-line. Regional end-systole was defined as the point of maximal wall thickness within 20 ms before peak negative LV dP/dt. Systolic wall thickening was calculated as end-systolic wall thickness minus end-diastolic wall thickness divided by the end-diastolic wall thickness.

Regional myocardial blood flow. Radiolabeled microspheres (15 μm diameter, ^{141}Ce, ^{114}In, ^{51}Cr, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc; NEN, Du Pont, Boston, Massachusetts) were injected into the coronary perfusion circuit ($1–2 \times 10^5$ suspended in 1 ml saline) to determine the regional myocardial blood flow and its distribution throughout the LAD coronary artery perfusion bed (model 5912, Gammaszint BF 5300 Packard, Germany). This procedure for the determination of blood flow has been validated extensively (28). Blood flow to the tissue at the site of the ultrasonic crystals is reported, and this piece of tissue was divided into transmural thirds of approximately equal thickness. In addition, the averaged subendocardial blood flow (14 ± 5 pieces with a weight of 1.00 ± 0.36 g) to the entire LAD-perfused territory was measured and related to myocardial infarct size.

Regional myocardial oxygen consumption. Myocardial oxygen consumption of the LAD-perfused territory was measured using anaerobically sampled blood drawn simultaneously from the coronary vein and an artery. Oxygen content was measured using a co-oximeter (Cavitron/LexO_2LexO2-Con-k,

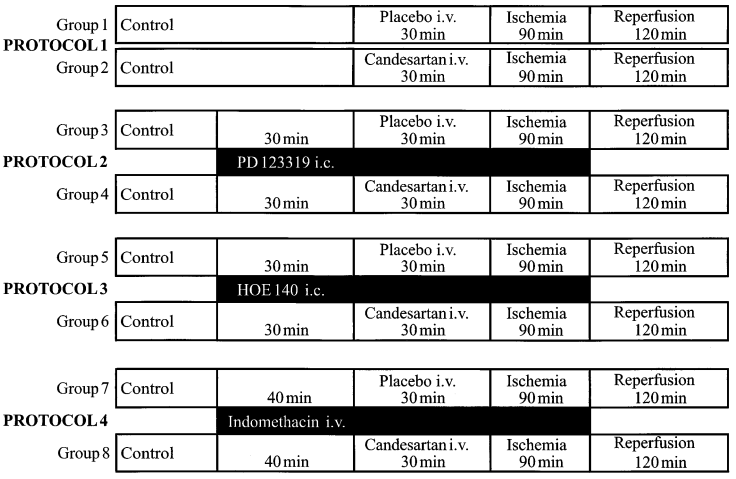

Figure 1. Schematic diagram of the four different experimental protocols.

Dr. B.G. Schlag, Bergisch Gladbach, Germany). Oxygen consumption of the anterior myocardial wall was calculated by multiplying the arterial-coronary venous oxygen difference by the transmural blood flow at the crystal site, as measured using the microsphere technique.

Morphology. At the end of each study, the heart was removed and sectioned from base to apex into five transverse slices in a plane parallel to the atrioventricular groove. The tissue slices were immersed in a 0.09 mol/liter sodium phosphate buffer (pH 7.4) containing 1.0% triphenyl tetrazolium chloride (TTC; Sigma, Deisenhofen, Germany) and 8% dextran (mol wt, 77.800) for 20 min at 37°C to identify infarcted tissue. The amount of infarcted tissue is expressed as a percentage of the LV area at risk, as determined by the microspheres technique (29).

Experimental protocols. Four subsequent protocols with two groups each were performed (Fig. 1). Each observation period began with the simultaneous withdrawal of pairs of arterial and coronary venous blood samples (1 ml per sample) for the measurement of oxygen content. During the blood sampling, microspheres were injected into the LAD perfusion system for the measurement of regional myocardial blood flow. Coronary arterial pressure, systemic hemodynamic and regional myocardial dimension data were continuously recorded to ensure that parameters were unaffected by the microsphere injection. Measurements were obtained within 2 min; these were performed under control conditions, after each drug administration and at 5 and 90 min following the reduction in coronary blood flow. Coronary blood flow was decreased with the roller pump to a level sufficient to reduce regional systolic wall thickening of the anterior wall by >90%. This adjustment period lasted approximately 3 min; thereafter, no further changes in flow were made. This level of hypoperfusion was maintained for 90 min. Following 90 min of ischemia, the myocardium was reperfused for 120 min to facilitate the identification of necrotic tissue. During reperfusion, coronary inflow was set to maintain a minimum coronary arterial pressure above 70 mm Hg.

Protocol 1. *Group 1: Placebo + 90-min severe ischemia (n = 9).* In the placebo-group following control measurements, a 40-ml saline solution was infused intravenously (i.v.) over 30 min.

Group 2: Candesartan + 90-min severe ischemia (n = 7). Following control measurements, candesartan [2-ethoxy-1[[2′-(1H-tetrazol-5-yl)biphenyl-4-yl]methy]-1H-benzimidazole-7-carboxylic acid] at a dose of 1 mg/kg (dissolved in 40 ml saline solution) was infused i.v. within 30 min. Left ventricular (LV) peak pressure decreased with candesartan and was adjusted to predrug values by aortic banding. Measurements were repeated 30 min following the administration of candesartan at matched LV peak pressure, before coronary blood flow was reduced. In five preliminary experiments, this dose of 1 mg/kg candesartan blocked the vasoconstriction induced by infusion of angiotensin II (15 μg/min intracoronarily, i.c.). Prior to candesartan, mean coronary arterial pressure and LV peak pressure increased with angiotensin II from 105 ± 1 to 126 ± 4 mm Hg and from 73 ± 3 to 103 ± 6 mm Hg, respectively (both $p < 0.05$). After treatment with candesartan, mean coronary arterial pressure (106 ± 4 vs. 107 ± 4 mm Hg) and LV peak pressure (70 ± 3 vs. 72 ± 3 mm Hg) did not change during infusion of angiotensin II.

Protocol 2. *Group 3: PD123319 + 90-min severe ischemia (n = 8).* Following control measurements, PD123319 (S)-1-[[4-(dimethylamino)-3-methylphenyl]methyl]-5-(diphenyl-acetyl)-4,5,6,7-tetrahydro-1H-imidazol[4,5-c]-pyridine-6-carboxylic acid] was infused at a dose (3 μg/kg/min i.c.) adapted from a previous report (30). Thirty minutes after the start of the PD123319 infusion, measurements were repeated, before coronary blood flow was reduced. Infusion of PD123319 was terminated at the end of ischemia.

Group 4: PD123319 + candesartan + 90-min severe ischemia (n = 8). Following control measurements, the infusion of PD123319 (3 μg/kg/min i.c.) was started. Following 30-min PD123319 infusion, candesartan (1 mg/kg i.v.) was infused. After a total of 60 min, measurements were repeated, before coronary blood flow was reduced. One animal of this group

died during ischemia due to irreversible ventricular fibrillation; data of this animal were excluded from the analysis.

Protocol 3. *Group 5: HOE140 + 90-min severe ischemia (n = 8).* Following control measurements, bradykinin B_2-receptors were blocked by infusion of HOE140 (0.01 μg/kg/min i.c.). The infusion was started 30 min prior to ischemia and then maintained until the end of ischemic period. This dose regimen has previously been demonstrated to abolish the coronary vasodilation induced by intracoronary bradykinin in the same porcine preparation (31). Thirty minutes after the start of the HOE140 infusion, measurements were repeated, before coronary blood flow was reduced.

Group 6: HOE140 + candesartan + 90-min severe ischemia (n = 7). Following control measurements, the infusion of HOE140 (0.01 μg/kg/min i.c.) was started. Following 30-min HOE140 infusion, candesartan (1 mg/kg i.v.) was infused. After a total of 60 min, measurements were repeated, before coronary blood flow was reduced.

Protocol 4. *Group 7: Indomethacin + 90-min severe ischemia (n = 8).* Following control measurements, the cyclooxygenase was inhibited by infusion of indomethacin (10 mg/kg i.v.). This dose regimen has previously been demonstrated to abolish the coronary vasodilation induced by intracoronary arachidonic acid (25). The infusion of indomethacin was started 40 min prior to ischemia. Fourty minutes after the start of the indomethacin infusion, measurements were repeated, before coronary blood flow was reduced.

Group 8: Indomethacin + candesartan + 90-min severe ischemia (n = 9). Following control measurements, the infusion of indomethacin (10 mg/kg i.v.) was started. Following a 40-min indomethacin infusion, candesartan (1 mg/kg i.v.) was infused. After a total of 70 min, measurements were repeated, before coronary blood flow was reduced. One animal of this group died during ischemia due to irreversible ventricular fibrillation; data of this animal were excluded from the analysis.

Data analysis and statistics. Hemodynamic data were recorded on an 8-channel recorder (Gould MK 200A, Cleveland, Ohio) and stored directly to the hard disk of an AT-type computer. Hemodynamic and functional parameters were digitized and recorded over a 20-s period during each microsphere injection (approximately 33 consecutive beats over at least two complete respiratory cycles) using CORDAT II software (32). Hemodynamic parameters reported are heart rate, LV peak pressure, LV dP/dt_{max} and mean coronary arterial pressure. Regional wall function is expressed as systolic wall thickening. Calculation of all hemodynamic parameters was done on a beat-to-beat basis, and data were then averaged. In addition, regional myocardial blood flow and oxygen consumption were measured.

Statistical analysis was performed using SYSTAT software (Urbana, Illinois). Data on hemodynamics, wall thickening, myocardial blood flow and oxygen consumption were compared using a two-way analysis of variance (ANOVA) for repeated measures, accounting for the different time points throughout the protocol and the eight groups of pigs. When significant differences were detected, individual mean values were compared using LSD post-hoc tests. Area at risk and infarct size were compared for each protocol separately by the Student unpaired t test. All data are reported as mean values ± standard error of the mean (SEM), and a p value less than 0.05 was accepted as indicating a significant difference in mean values. Linear regression analyses between subendocardial blood flow at 5 min ischemia in the LV area at risk and infarct size (expressed as percentage of the area at risk) were performed in all groups. Regression lines were compared by analysis of covariance (ANCOVA).

Results

No significant differences were observed in any measured parameter among the groups under control conditions. Data on systemic hemodynamics are summarized in Table 1. Data on regional myocardial function, blood flow and oxygen consumption are summarized in Table 2. The incidence of arrhythmias was low (peak incidence of 18 ± 9 ventricular extrasystoles/5 min in the placebo group) and not different among groups.

In all groups of pigs, by decreasing the pump speed, coronary blood flow was reduced such that mean coronary arterial pressure was decreased. The LV peak pressure was decreased at 5 min ischemia in groups 1, 2, 4, 5 and 6. The $LVdP/dt_{max}$ was decreased at 5 min ischemia in all groups of pigs with the exception of group 3. In all groups of pigs, both systolic wall thickening of the anterior myocardium, subendocardial and transmural myocardial blood flow and regional myocardial oxygen consumption were decreased at 5 min ischemia. During the remainder of the 90-min ischemic period, systemic hemodynamics, anterior systolic wall thickening, regional myocardial blood flow and oxygen consumption did not change further.

Left ventricular peak pressure decreased with candesartan from 97 ± 2 to 86 ± 5 mm Hg and was readjusted before further measurements. Candesartan had no significant effect on heart rate, $LVdP/dt_{max}$ (Table 1), systolic wall thickening of the anterior myocardium or regional myocardial oxygen consumption (Table 2). Transmural myocardial blood flow increased with candesartan in group 6.

PD123319 alone and in combination with candesartan did not significantly change systemic hemodynamics, anterior systolic wall thickening and oxygen consumption (Tables 1 and 2). Subendocardial and transmural myocardial blood flow tended to increase (NS) with PD123319.

Both alone and in combination with candesartan, HOE140 did not significantly change systemic hemodynamics, anterior systolic wall thickening, regional myocardial blood flow and oxygen consumption (Tables 1 and 2).

Indomethacin increased LV peak pressure and mean coronary arterial pressure, whereas anterior systolic wall thickening was decreased. Indomethacin alone and in combination with candesartan did not significantly change regional myocardial blood flow and oxygen consumption (Tables 1 and 2).

Table 1. Systemic Hemodynamics

	Group	Control	Drug	Drug + Candesartan	5-min Ischemia	90-min Ischemia
HR (min^{-1})	1	103 ± 2			105 ± 3	105 ± 3
	2	95 ± 5		98 ± 5	101 ± 5	104 ± 7
	3	96 ± 3	96 ± 3		103 ± 6	105 ± 4
	4	94 ± 3	95 ± 3	96 ± 4	100 ± 2	110 ± 8
	5	99 ± 3	100 ± 3		101 ± 3	104 ± 2
	6	95 ± 3	95 ± 4	97 ± 4	98 ± 4	107 ± 4
	7	102 ± 6	103 ± 7		104 ± 6	116 ± 7
	8	102 ± 4	102 ± 3	108 ± 4	110 ± 4	115 ± 7
LVpP (mm Hg)	1	93 ± 3			84 ± 5*	81 ± 4*
	2	97 ± 2		94 ± 3	79 ± 3*#	84 ± 5*
	3	94 ± 5	98 ± 4		83 ± 4	85 ± 8
	4	100 ± 5	103 ± 6	101 ± 5	86 ± 5#	82 ± 6*
	5	100 ± 3	97 ± 3		82 ± 3*#	82 ± 3*
	6	98 ± 5	100 ± 5	93 ± 5	81 ± 4*	83 ± 5*
	7	92 ± 4	106 ± 5*		83 ± 4#	89 ± 4
	8	98 ± 4	112 ± 6*	105 ± 6	91 ± 5§	88 ± 5
$LVdP/dt_{max}$ (mm Hg/s)	1	1319 ± 67			919 ± 64*	949 ± 56*
	2	1373 ± 95		1244 ± 77	883 ± 53*#	1083 ± 104*
	3	1241 ± 113	1272 ± 102		1017 ± 92	1067 ± 131
	4	1319 ± 64	1356 ± 73	1274 ± 103	994 ± 78*#	1074 ± 110*
	5	1293 ± 78	1274 ± 110		899 ± 41*#	950 ± 57*
	6	1336 ± 73	1337 ± 68	1163 ± 54	918 ± 39*#	947 ± 48*
	7	1299 ± 84	1140 ± 81		909 ± 42*#	983 ± 76*
	8	1452 ± 98	1274 ± 70	1229 ± 55*	1024 ± 36*#	1038 ± 90*
CAP (mm Hg)	1	120 ± 2			29 ± 2*#	26 ± 2*
	2	114 ± 3		118 ± 2	27 ± 1*#	25 ± 1*
	3	116 ± 4	119 ± 5		27 ± 3*#	23 ± 3*
	4	120 ± 5	116 ± 2	112 ± 4	29 ± 2*#	27 ± 2*
	5	115 ± 1	115 ± 2		29 ± 1*#	28 ± 2*
	6	113 ± 3	111 ± 3	112 ± 4	27 ± 1*#	24 ± 1*
	7	117 ± 3	158 ± 8*		29 ± 2*#	27 ± 2*
	8	119 ± 4	154 ± 9*	129 ± 10#	30 ± 1*#	28 ± 2*

HR, heart rate; LVpP, left ventricular peak pressure; LV dP/dt_{max}, maximum of the first derivative of left ventricular pressure; CAP, mean coronary arterial pressure; *$p < 0.05$ vs. control; #$p < 0.05$ vs. preceding value; §$p < 0.05$ vs. drug. Group 1, placebo; group 2, candesartan; group 3, PD123319; group 4, PD123319 + candesartan; group 5, HOE140; group 6, HOE140 + candesartan; group 7, indomethacin; group 8, indomethacin + candesartan.

Infarct size. The area at risk in percent of LV mass was comparable among all groups of pigs (group 1: 46.8 ± 3.0%; group 2: 55.3 ± 1.9%; group 3: 47.4 ± 2.8%; group 4: 50.9 ± 3.1%; group 5: 49.9 ± 3.1%; group 6: 44.5 ± 2.6%; group 7: 44.1 ± 3.2%; group 8: 45.1 ± 3.5%).

In placebo pigs, following 90 min of ischemia and 120 min of reperfusion, infarct size was 21.8 ± 4.8% of the area at risk (Fig. 2). Candesartan significantly reduced infarct size to 9.7 ± 2.5% ($p < 0.05$ vs. placebo) (Fig. 2). Infarct size for any given subendocardial blood flow was significantly reduced in the candesartan group ($y = -210.1 \cdot \times + 18.6$, $n = 7$, $r = -0.83$) vs. placebo group ($y = -497.4 \cdot \times + 44.7$, $n = 9$, $r = -0.84$, $p < 0.05$) (Fig. 2).

The AT_2-receptor antagonist PD123319 per se did not affect infarct size (16.8 ± 4.0%) (Fig. 3). Pretreatment with PD123319 abolished the reduction of infarct size by candesartan (23.2 ± 4.7%). The relationships between infarct size and subendocardial blood flow in groups 3 and 4 were superimposable ($y = -430.0 \cdot \times + 36.5$, $n = 8$, $r = -0.85$ vs. $y = -491.7 \cdot \times + 47.0$, $n = 7$, $r = -0.90$) (Fig. 3).

The B_2-receptor antagonist HOE140 per se did not affect infarct size (15.7 ± 2.8%) (Fig. 4), but pretreatment with HOE140 abolished the reduction of infarct size by candesartan (18.2 ± 4.0%). The relationships between infarct size and subendocardial blood flow in groups 5 and 6 were superimposable ($y = -328.9 \cdot \times + 33.0$, $n = 8$, $r = -0.86$ vs. $y = -294.9 \cdot \times + 33.1$, $n = 7$, $r = -0.88$) (Fig. 4).

The cyclooxygenase inhibitor indomethacin per se did not affect infarct size (22.5 ± 5.5%) (Fig. 5), but pretreatment with indomethacin abolished the reduction of infarct size by candesartan (21.1 ± 5.2%). The relationships between infarct size and subendocardial blood flow in groups 5 and 6 were superimposable ($y = -370.7 \cdot \times + 38.8$, $n = 8$, $r = -0.81$ vs. $y = -323.1 \cdot \times + 34.8$, $n = 8$, $r = -0.74$) (Fig. 5).

Table 2. Regional Myocardial Function, Blood Flow and Oxygen Consumption

	Group	Control	Drug	Drug + Candesartan	5-min Ischemia	90-min Ischemia
WT (%)	1	42.4 ± 6.3			−1.5 ± 0.9*#	1.4 ± 2.3*
	2	40.9 ± 6.7		40.5 ± 7.8	0.4 ± 1.2*#	1.5 ± 1.1*
	3	40.0 ± 3.3	39.6 ± 3.0		0.1 ± 1.0*#	0.2 ± 0.8*
	4	42.3 ± 4.3	40.3 ± 4.9	39.1 ± 4.7	−1.5 ± 1.5*#	−0.2 ± 1.4*
	5	36.0 ± 4.5	28.8 ± 3.4		1.2 ± 1.3*#	2.0 ± 0.9*
	6	38.5 ± 5.5	39.5 ± 4.5	35.0 ± 4.2	0.3 ± 2.1*#	1.7 ± 2.1*
	7	36.5 ± 3.1	27.8 ± 2.6*		−1.0 ± 1.2*#	0.3 ± 2.2*
	8	40.2 ± 4.5	31.0 ± 5.6	29.3 ± 4.5	−1.6 ± 1.6*#	0.0 ± 1.9*
mTMF ($ml \cdot min^{-1} \cdot g^{-1}$)	1	0.77 ± 0.05			0.11 ± 0.02*#	0.11 ± 0.02*
	2	0.81 ± 0.04		0.87 ± 0.09	0.16 ± 0.01*#	0.16 ± 0.02*
	3	0.75 ± 0.05	0.97 ± 0.16		0.13 ± 0.02*#	0.14 ± 0.03*
	4	0.86 ± 0.06	1.01 ± 0.11	1.08 ± 0.11*	0.15 ± 0.04*#	0.15 ± 0.03*
	5	0.71 ± 0.07	0.77 ± 0.06		0.12 ± 0.01*#	0.13 ± 0.01*
	6	0.69 ± 0.01		0.79 ± 0.04*	0.09 ± 0.02*#	0.12 ± 0.03*
	7	0.79 ± 0.10	0.78 ± 0.09		0.10 ± 0.02*#	0.10 ± 0.02*
	8	0.76 ± 0.04	0.75 ± 0.04	0.81 ± 0.06	0.14 ± 0.03*#	0.16 ± 0.04*
ENDO ($ml \cdot min^{-1} \cdot g^{-1}$)	1	0.70 ± 0.05			0.06 ± 0.02*#	0.05 ± 0.01*
	2	0.92 ± 0.08		0.89 ± 0.10	0.05 ± 0.02*#	0.06 ± 0.02*
	3	0.69 ± 0.05	0.81 ± 0.10		0.05 ± 0.01*#	0.04 ± 0.01*
	4	0.74 ± 0.04	0.92 ± 0.11	0.91 ± 0.08*	0.07 ± 0.02*#	0.06 ± 0.02*
	5	0.70 ± 0.07	0.87 ± 0.10		0.05 ± 0.01*#	0.06 ± 0.01*
	6	0.66 ± 0.05		0.79 ± 0.08	0.04 ± 0.02*#	0.04 ± 0.01*
	7	0.89 ± 0.13	0.86 ± 0.13		0.05 ± 0.01*#	0.04 ± 0.01*
	8	0.78 ± 0.03	0.76 ± 0.06	0.82 ± 0.04	0.05 ± 0.02*#	0.07 ± 0.03*
$M\dot{V}\ O_2$ ($\mu l \cdot min^{-1} \cdot g^{-1}$)	1	76.7 ± 6.4			14.6 ± 3.2*#	14.3 ± 4.2*
	2	78.0 ± 5.5		73.9 ± 8.8	18.7 ± 2.0*#	18.3 ± 2.5*
	3	60.9 ± 4.7	59.5 ± 4.6		14.7 ± 2.9*#	13.3 ± 2.2*
	4	70.3 ± 5.2	78.6 ± 8.2	77.2 ± 6.4	16.8 ± 4.3*#	15.9 ± 4.2*
	5	68.8 ± 4.5	66.7 ± 8.1		15.8 ± 1.7*#	16.8 ± 1.9*
	6	66.9 ± 2.1		62.6 ± 2.4	11.0 ± 2.4*#	10.4 ± 3.0*
	7	75.2 ± 3.6	74.9 ± 3.6		12.1 ± 2.2*#	11.7 ± 1.4*
	8	74.2 ± 4.5	75.5 ± 4.9	78.1 ± 6.6	16.2 ± 4.4*#	17.1 ± 3.9*

WT, anterior systolic wall thickening in percent of the end-diastolic wall thickness; mTMF, mean transmural myocardial blood flow; ENDO, subendocardial blood flow; $M\dot{V}\ O_2$, regional myocardial oxygen consumption; *$p < 0.05$ vs. control; #$p < 0.05$ vs. preceding value. Group 1, placebo group 2, candesartan; group 3, PD123319; group 4, PD123319 + candesartan; group 5, HOE; group 6, HOE + candesartan; group 7, indomethacin; group 8, indomethacin + candesartan.

Discussion

In the present study, candesartan reduced infarct size, and this beneficial effect was not related to favorable changes in heart rate, LV peak pressure and blood flow. The infarct size reduction by candesartan was abolished with blockade of either the angiotensin II AT_2-receptor or the bradykinin B_2-receptor or by cyclooxygenase inhibition, suggesting a cardioprotective action of candesartan through a signal cascade of AT_2-receptor activation, bradykinin and prostaglandins.

Critique of methods. Infarct size as determined by TTC-staining after 90 min of ischemia and 2 h of reperfusion was one major end point of the present study. Although the validity of TTC-staining to identify myocardial necrosis within the time frame of 90 min of ischemia and 2 h of reperfusion has not been rigorously confirmed by electron microscopy, numerous studies from different laboratories have used TTC-staining to delineate myocardial necrosis within such time frame of ischemia/reperfusion (33,34).

Infarct size with PD123319 (group 3: 17 ± 4%) and HOE140 (group 5: 16 ± 3%) was slightly—although not significantly—less than with placebo (group 1: 22 ± 5%). We do not have any methodological or mechanistic explanation other than that this slight reduction may be related to the relatively small number of animals per group.

Pigs were used in the present study because their coronary anatomy (18), extent of collateral flow (19) and time course of infarct development most closely resemble that observed in humans (20). In pigs, complete occlusion of the LAD coronary artery results in a high incidence of ventricular fibrillation (45% within 20 min) (35) and extensive infarction of the left ventricle with subsequent pump failure. Therefore, in the present study, the LAD perfusion territory was hypoperfused at low but maintained flow, resulting in a large area at risk (49% of the LV mass on the average), but a small infarct size when expressed as a percent of the area at risk (22 ± 5% in group 1). However, infarct size expressed as a percent of the

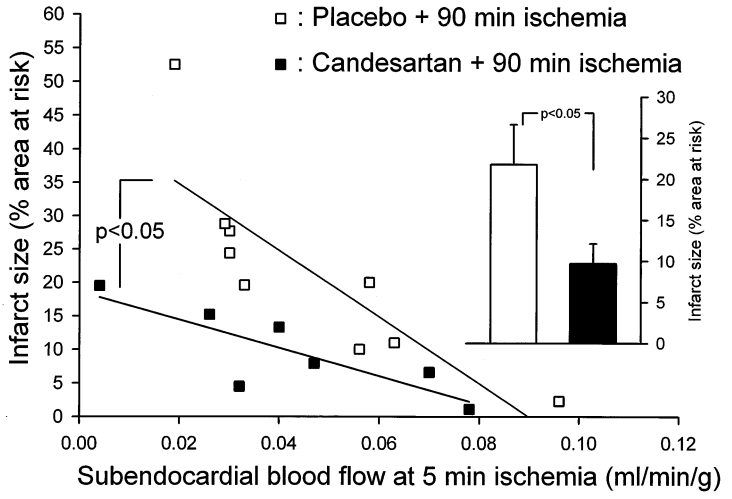

Figure 2. Effect of candesartan on infarct size. Infarct size in percent of the area at risk was significantly reduced in the pigs receiving candesartan (**filled bars**) as compared to that of the placebo group (**open bars**) ($p < 0.05$ vs. placebo). In the relationships between infarct size and subendocardial blood flow, subendocardial blood flow in the area at risk correlated inversely to infarct size in the placebo (**open squares**) and the candesartan group (**filled squares**). Infarct size for any given subendocardial blood flow was significantly reduced in the candesartan group.

total LV mass in the present study averaged 11 ± 3% in the placebo group and was thus even somewhat higher than that in a previous study using pigs with a total occlusion of only one distal LAD branch (7%) (36).

Many patients suffering from coronary artery disease over prolonged periods of time develop an extensive collateral circulation (37). In this scenario, an acute total occlusion of one coronary artery will result in low-flow rather than no-flow ischemia in the dependent myocardium. Technically, perfusion at low flow in the present study permitted the delivery of the AT_2-receptor antagonist PD123319 and the bradykinin B_2-receptor antagonist HOE140 throughout the sustained ischemia to maximize the local effect without influencing systemic hemodynamics. Also, low-flow hypoperfusion allowed us to relate infarct size to ischemic subendocardial blood flow. Ischemic blood flow is, apart from the size of the area at risk, the major determinant of infarct development (38), and therefore the relation of infarct size to subendocardial blood flow is a more sensitive end point than infarct size per se.

In the present study, candesartan was used only at a single dose of 1 mg/kg i.v. (25 to 40 mg total dose), which was derived from studies in rats (39,40) and dogs (3,39). In patients with hypertension, the prodrug candesartan cilexetil is given in oral doses of 8 to 16 mg (41). This dose results in plasma concentrations of candesartan (21) comparable to those measured in dogs with 1 mg/kg i.v. infusion of candesartan (39,42). Thus, the dose of candesartan used in the present study appears to be clinically relevant, as also reflected by a similar decrease in systemic blood pressure (41).

AT_1-receptor blockade and infarct size. When given 24 h following the onset of ischemia in rat hearts, AT_1-receptor-blockade with losartan (43) and candesartan cilexetil (44), the prodrug of candesartan, did not affect infarct size. Also, pretreatment with losartan had no significant effect on infarct size in rats (11–13) and rabbits (14–16). Pretreatment with EXP3174, the active metabolite of losartan, did not limit infarct size resulting from 90-min left circumflex coronary artery (LCX) occlusion and 4-h reperfusion in anesthetized dogs (17), whereas in pigs, EXP3174 reduced infarct size resulting from 1-h LAD occlusion and 2 h reperfusion signif-

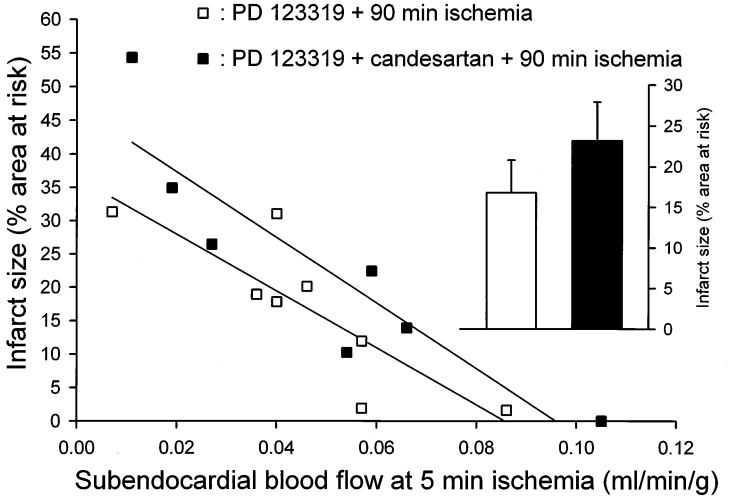

Figure 3. Effect of candesartan on infarct size during AT_2-receptor blockade. Infarct size in percent of the area at risk was not different in the pigs receiving PD123319 + candesartan (**filled bars**) as compared to that of the PD123319 group (**open bars**). The relationships between infarct size and subendocardial blood flow between the two groups were superimposable.

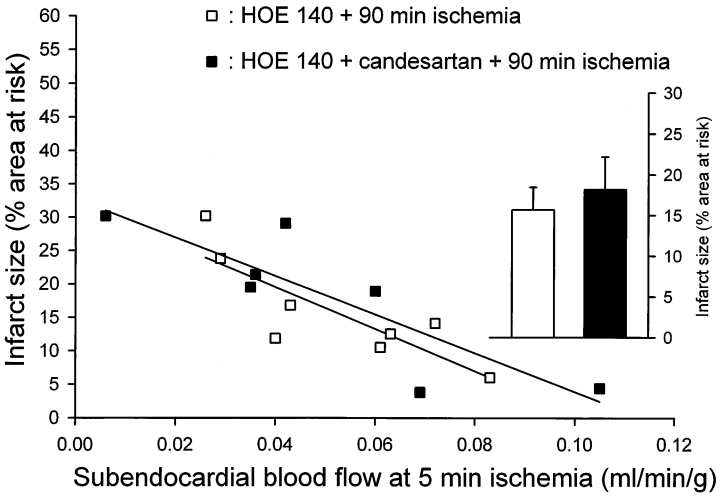

Figure 4. Effect of candesartan on infarct size during bradykinin B_2-receptor blockade. Infarct size in percent of the area at risk was not different in the pigs receiving HOE140 + candesartan **(filled bars)** as compared to that of the HOE140-group **(open bars)**. The relationships between infarct size and subendocardial blood flow between the two groups were superimposable.

icantly from 71 ± 13% to 35 ± 17% (10). One possible explanation for the difference between the two latter studies may be the dosage of EXP3174, which was 0.1 mg/kg i.v. in dogs (17) and 1 mg/kg i.v. in pigs (10). In the present study, the active metabolite candesartan, also at a dose of 1 mg/kg i.v., reduced infarct size from 22 ± 5% in placebo pigs to 10 ± 3% ($p < 0.05$, Fig. 2).

Candesartan and AT$_2$-receptor activation. Cardiovascular effects of angiotensin II (i.e., vasoconstriction and ventricular hypertrophy), have been mainly attributed to AT$_1$-receptor activation (1), whereas the AT$_2$-receptor mediates the inhibition of cell proliferation in rat coronary endothelial cells (45). More than 80% of the angiotensin II receptors in both atrial and LV preparations of the normal pig heart are AT$_1$-receptors (46). In human hearts, the predominant AT-receptor subtype remains controversial. In membrane preparations from normal human left ventricles, the ratio of AT$_1$- to AT$_2$-receptors was either reported as 62%:38% (47) or as 29%:71% (48).

Results of the present study suggest that angiotensin II—in the face of AT$_1$-receptor blockade—exerts cardioprotective effects via AT$_2$-receptor activation (Fig. 3). One potential—although purely speculative—explanation for the lack of angiotensin II to mediate cardioprotection without AT$_1$-receptor blockade would be that even elevated angiotensin II concentrations during ischemia (2) and following acute myocardial infarction (49,50) may not be sufficient to activate enough AT$_2$-receptors. Indeed, PD123319 per se did not significantly alter infarct size (Fig. 3), indicating that there was no significant AT$_2$-receptor mediated effect in the absence of AT$_1$-receptor blockade. In accordance with our findings, PD123319 had no effect on LV remodelling and function in rats with myocardial infarction, but did abolish the beneficial effects on LV remodelling achieved by the AT$_1$-receptor antagonist L-158809 (9). Only increased levels of angiotensin II in the presence of an AT$_1$-receptor antagonist (21), as well as the redistribution of angiotensin II toward activation of AT$_2$-receptors, appear to induce cardioprotection.

Although PD123319 did not alter infarct size in the present study, it has been reported to increase global LV function

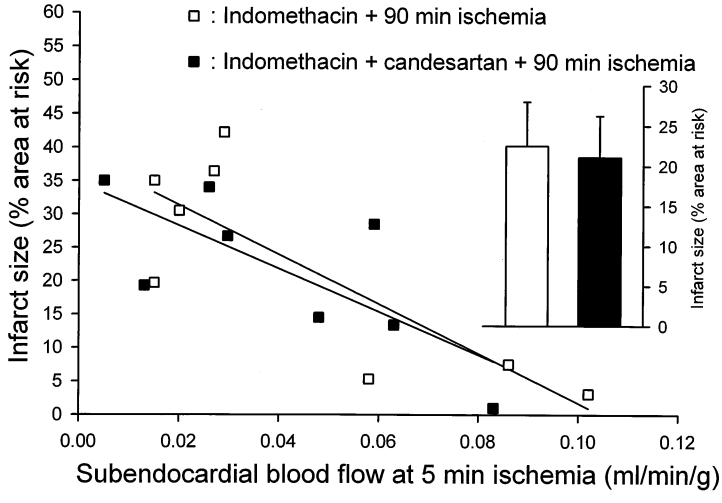

Figure 5. Effect of candesartan on infarct size during cyclooxygenase inhibition. Infarct size in percent of the area at risk was not different in the pigs receiving indomethacin + candesartan **(filled bars)** as compared to that of the indomethacin group **(open bars)**. The relationships between infarct size and subendocardial blood flow between the two groups were superimposable.

during reperfusion following 30-min global ischemia in isolated rat hearts (51); this increase in contractile function during reperfusion, may, however, reflect an AT_1-receptor-mediated positive inotropic effect (52) after redistribution of angiotensin II toward activation of AT_1-receptors.

Candesartan and bradykinin. In isolated dog coronary arteries, AT_2-receptor activation induces the activation of a local kallikrein-kinin system and generation of bradykinin (22). The protective effects of AT_1-receptor blockade on ventricular fibrillation in isolated ischemic rat hearts are abolished by blockade of the bradykinin B_2-receptor (7). Also, in rats with heart failure secondary to myocardial infarction, the beneficial effects of AT_1-receptor blockade on LV remodelling and function are abolished by the B_2-receptor antagonist HOE140 (9). In accordance, in the present study, the infarct size-limiting effect of candesartan was also abolished by pretreatment with the bradykinin B_2-receptor antagonist HOE140 (Fig. 4). Interestingly, the beneficial effects of ACE-inhibitors on infarct size in rats (11), rabbits (53) and dogs (2) and on myocardial stunning in dogs (25) are also mediated by bradykinin.

Candesartan and prostaglandins. The AT_1-receptor antagonist losartan induces prostacyclin release in porcine aortic smooth muscle cells (54). In accordance, in the present study, the infarct size-limiting effect of candesartan was also abolished by pretreatment with the cyclooxygenase inhibitor indomethacin (Fig. 5), suggesting that prostaglandins mediate the cardioprotection afforded by AT_1-receptor blockade. Indeed, stimulation of endogenous prostacyclin release reduces infarct size (55) and improves the contractile recovery from myocardial stunning (56) in pigs. Also, the beneficial effects of the ACE-inhibitor ramiprilat on infarct size in rats (11) and on myocardial stunning in dogs (25) are mediated by prostaglandins.

Clinical implications. In patients with myocardial infarction, cardioprotective effects of ACE-inhibitors have been documented in large clinical trials (57,58). However, some patients discontinue therapy with ACE-inhibitors because of adverse experiences, whereas AT_1-receptor antagonists appear to be better tolerated (59). When added to the preexisting treatment of heart failure in patients, AT_1-receptor blockade decreases mortality to a greater extent than does additional ACE-inhibitor treatment (59). Moreover, combined ACE-inhibition and AT_1-receptor antagonism may act in an additive manner to reduce systemic vascular resistance in humans (60) and improve LV function in pigs with chronic heart failure (46).

With reference to the infarct size reduction by candesartan observed in the present study, patients under treatment with AT_1-receptor blocker for indications such as hypertension and ventricular remodelling after myocardial infarction are likely to have improved prognosis when suffering an acute myocardial infarction.

Conclusions. In the present study, the angiotensin II AT_1-receptor antagonist candesartan exerted a cardioprotective action that was not related to more favorable systemic hemodynamics or regional myocardial blood flow, but was mediated through a signal cascade of AT_2-receptor activation, bradykinin and prostaglandins.

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